IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

CUBIST PHARMACEUTICALS, INC., Plaintiff,	)	C.A. No. 12-367-GMS (CONSOLIDATED)
v.	)	
HOSPIRA, INC., Defendant.	)	

DECLARATION OF WILLIAM GERWICK, PH.D.

I, William Gerwick, Ph.D. hereby declare as follows:

I. INTRODUCTION

1. As set forth more fully below, I have education, training and experience in the field of natural products, including how products like daptomycin can be made from fermentation of bacteria. I have been asked to provide background information regarding natural products, including how natural products are made by fermentation and how the properties of natural products can be determined. Such information would have been known to a person of ordinary skill in the art reviewing U.S. Patent Nos. 8,058,238 ("the '238 patent") and 8,129,342 ("the '342 patent") (collectively, the "high purity patents").

II. QUALIFICATIONS

2. I received my Bachelor of Science in Biochemistry from University of California at Davis in 1976. In 1981, I earned my Ph.D. in Oceanography, with an emphasis on Marine Organic Chemistry, from University of California, San Diego. My graduate research focused on the discovery of novel natural products. I worked at the University of Connecticut School of

Pharmacy as a postdoctoral research associate researching the biosynthesis of microbial antibiotics produced by Gram-positive soil bacteria of the genus Streptomyces.

3. I was Professor in the College of Pharmacy at Oregon State University for 21 years. I conducted research on anti-cancer and antibiotic compounds produced by diverse microorganisms. During these studies, we discovered numerous cyclic peptides (*i.e.*, amino acids that are linked together with bonds known as peptide bonds to form circular chains) produced by cyanobacteria with antibiotic and anti-cancer activity. We isolated these compounds, developed methods for analyzing their structure, and determined the stereochemistry of these compounds using a variety of techniques.

4. Currently, I am a Professor of Pharmaceutical Sciences at Skaggs School of Pharmacy and Pharmaceutical Sciences at the University of California, San Diego. I am also a Professor of Oceanography at Scripps Institution of Oceanography, at the University of California, San Diego. Further, I am a member of the Moores Cancer Center at the University of California, San Diego, have an affiliate professorship at the University of Aberdeen, Scotland, and have a Research Scientist position at the Smithsonian Tropical Research Institute in Panama.

5. Since receiving my doctoral degree, I have discovered, isolated, elucidated the structure of, and biologically characterized new anti-cancer, antimicrobial, and anti-inflammatory or neurotoxic natural product compounds. I have also studied the biosynthetic origin of these compounds.

6. I have taught courses in Chemistry, Biochemistry, Pharmacy, Medicinal Chemistry, Pharmacology, Marine Natural Products Chemistry, Pharmacognosy (the study of

naturally occurring pharmaceutical agents), Structural Analysis of Organic Compounds, Marine Science, and Oceanography. Several of these courses have focused on methods of isolating and characterizing the structure of natural products, including cyclic peptides.

7. I am a named inventor on seventeen patents and patent applications, including inventions relating to the isolation, purification, and structure characterization of cyclic peptides with useful properties. My most recent patent concerns the discovery and characterization of a novel natural product that has potential to treat certain types of cancer.

8. I am the author or co-author of over 250 peer-reviewed scientific publications. Most of these concern the discovery, isolation, and characterization of new natural products. I have written twenty-five review papers and book chapters, several of which concern cyclic peptides from diverse microorganisms. For example, in 2000, I published a comprehensive review of the nitrogen-containing metabolites from marine bacteria, including many marine *Streptomyces*.

9. I was an Associate Editor of the Journal of Natural Products from 2001 to 2007. This journal, jointly published by the American Society of Pharmacognosy and the American Chemical Society, is one of the premier journals for publication of the isolation and characterization of new natural products. In this capacity, I oversaw the review of approximately 100 papers per year. Additionally, I am a frequent reviewer for the following journals: Journal of the American Chemical Society, Proceedings of the National Academy of Sciences, Journal of Organic Chemistry, Journal of Natural Products, and Organic Letters. I served as President for the American Society of Pharmacognosy in 2002 through 2003, and as a standing member of the

National Institutes of Health Study Section on Bioorganic and Natural Products Chemistry in 2003 through 2007. I am currently an invited member of the College of Scientific Reviewers for the National Institutes of Health ("NIH").

10. I have attached a copy of my curriculum vitae as Exhibit 1.

III. PERSON OF ORDINARY SKILL

11. I have read the high purity patents. They generally deal with highly purified daptomycin produced by fermentation and purification processes. Accordingly, I believe that one of ordinary skill in the art in the field of the inventions of the '238 and '342 patents would be familiar with techniques for making and characterizing natural products. That person would typically hold a degree in chemistry, biochemistry, chemical engineering, or complementary discipline and have laboratory experience in the manufacturing, purification, analysis, and/or characterization of pharmaceutical products for medicinal use.

IV. TECHNOLOGICAL BACKGROUND

A. FERMENTATION PRODUCTS

12. Many current pharmaceuticals derive from compounds produced in the natural world. Some of these products are made by microorganisms through a process called fermentation. Daptomycin is one such product, made by fermentation of the bacteria *Streptomyces roseosporus*. Fermentation is the process of growing cells in a nutrient-rich medium under specific conditions. Fermentation begins with placing a pure strain of a microorganism into a vessel of enriched growth medium. The microorganism is allowed to reproduce (*i.e.*, grow) for a sufficient time to convert the medium into the desired product. Once

the fermentation is complete, the desired product is isolated from the medium.

13. Fermentation is widely used in the pharmaceutical industry to produce valuable compounds such as active drug substances. Although compounds can be built one atom at a time using chemistry techniques referred to generally as "synthesis," fermentation is cheaper, faster, and more efficient, particularly for large peptides (*e.g.*, daptomycin).

B. STEREOCHEMISTRY

14. Every natural product molecule has a chemical structure, that is the 2-dimensional arrangement of the various atoms that make up the molecule, also known as a "planar structure." The chemical structure includes the various bonds connecting the atoms. Most natural product molecules also have stereochemistry, also known as the "stereostructure." The stereostructure of a molecule is the orientation of its atoms and bonds in 3-dimensions.

15. The stereostructure (also called chirality) of a molecule is determined by the left or right handed orientation of individual atoms in the molecule. Chiral carbon atoms are carbon atoms with four different substituents attached. Compounds with identical chemical structure but with different arrangements of substituents on a chiral carbon are called stereoisomers. In amino acids, these stereoisomers are commonly assigned D or L designation, where D usually means a right-handed arrangement of atoms around the chiral carbon (also known as an alpha carbon) and L usually means left-handed arrangement.[1] Most amino acids have a carbon with four different substituents attached and therefore can either be in a D or L configuration.

[1] Handedness is defined by assigning the substituents a priority according to a well-known set of rules known as Cahn-Ingold-Prelog Priority Rules, or CIP rules.

16. The past two decades have seen enormous improvements in the quality of techniques to characterize complicated molecules produced by fermentation, like daptomycin. However, from the 1980's through the early 2000's, techniques for determining the characteristics of natural products were not as refined, and mistakes in structure and stereochemistry were often made. For example, a review article on natural products reports that from January 1990 to April 2004 there were "well over 300 structural revisions, many of which extended far beyond simple stereochemical problems into the realm of profound, and sometimes complete, constitutional changes." Nicolau & Snyder, *Agnew. Chem. Int. Ed.*, 44:1012-1044 (2005) at 1015-1016 (Exhibit 2). In one area of natural products, marine natural products, there were over 40 corrections of stereochemistry from 2005 to 2010. Suyama et al., *Bioorg. Med. Chem.*, 19:6675-6701 (2011) at 6685-6690, Table 7 (Exhibit 3). Those of ordinary skill in the art were aware of these issues in the 1990's. *See, e.g.*, Nicolau & Snyder at 1013-1015 (describing history of natural product structure elucidation) and Table 1 (listing corrections to natural product structures determined in, *inter alia*, the 1990's).

17. Given that stereochemical corrections are so common, one skilled in the art from the early 1980's to the present would appreciate that the characterization of a natural product molecule can have errors. For example, a D amino acid could be originally misidentified as L and later corrected. One skilled in the art would not view such corrections as a fundamental change, because the natural product has not changed, but rather a clarification to the characterization of the natural product that more accurately reflects the true nature of the underlying molecule.

C. DAPTOMYCIN

18. Daptomycin's structure is "comprised of a decanoyl side chain linked to the N-terminal tryptophan of a cyclic 13-amino acid peptide (Fig. 1)." '238 patent, col. 1:58-63. Daptomycin essentially has three parts: (1) the cyclic region of the peptide; (2) the tail region of the peptide; and (3) the fatty acid chain. The cyclic and tail regions of the peptide together are composed of 13 amino acids, and the fatty acid chain is a straight chain of ten carbons referred to as "n-decanoyl." Figure 1 from the high purity patents (reproduced below) shows the two-dimensional chemical structure of daptomycin. Figure 2 below (which is not found in the high purity patents) represents daptomycin's chemical structure using a three-letter abbreviation for each amino acid and "R^N" to represent the fatty acid chain. Figure 2 also represents the three-dimensional structure of daptomycin with "D" or "L" designations referring to the stereochemistry of each amino acid.

Figure 1 of the high purity patents.

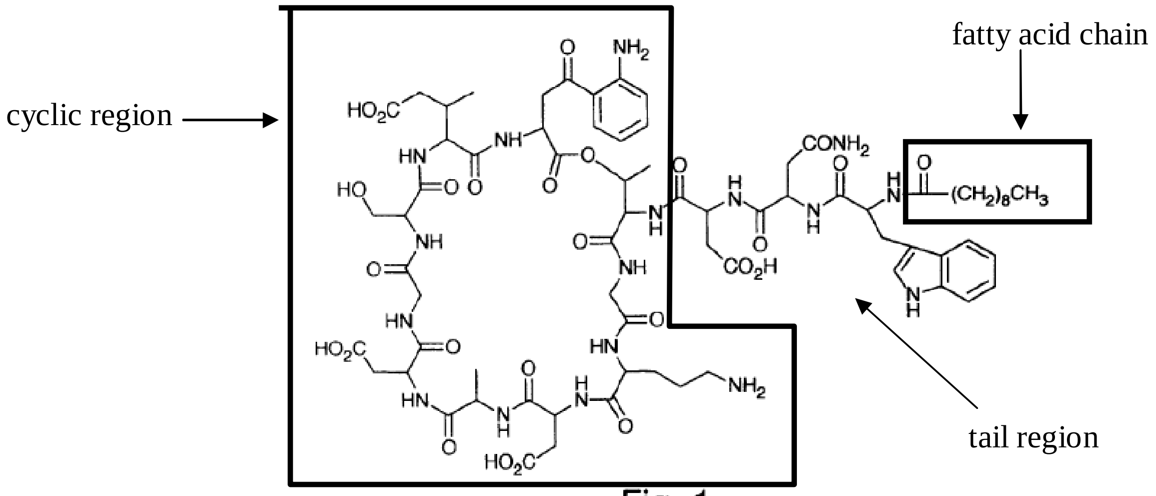
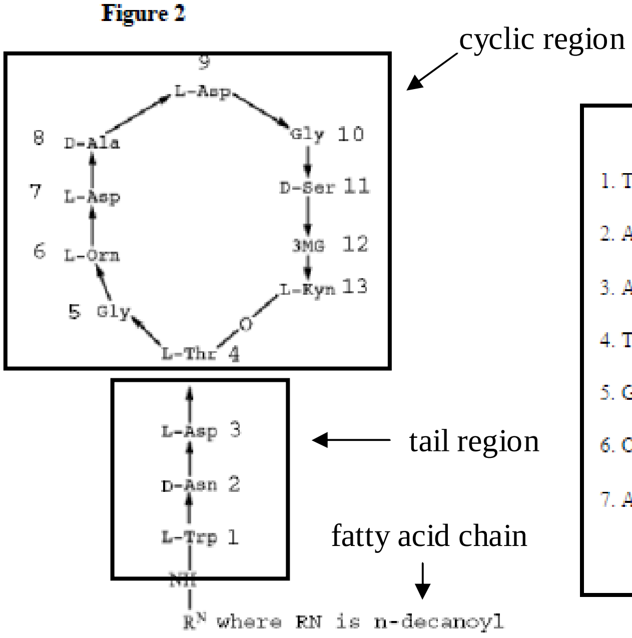


Fig. 1

Figure 2


cyclic region
9
L-Asp
8 D-Ala
Gly 10
7 L-Asp
D-Ser 11
6 L-Orn
3MG 12
L-Kyn 13
5 Gly
O
L-Thr 4
L-Asp 3
D-Asn 2
L-Trp 1
NH
tail region
fatty acid chain
R^N where RN is n-decanoyl

Amino Acid Residues for Figure 2

1. Trp (tryptophan)	8. Ala (alanine)
2. Asn (asparagine)	9. Asp (aspartic acid)
3. Asp (aspartic acid)	10. Gly (glycine)
4. Thr (threonine)	11. Ser (serine)
5. Gly (glycine)	12. 3-MG (L-threo-3-methylglutamic acid)
6. Orn (ornithine)	13. Kyn (kynurenine)
7. Asp (aspartic acid)	

19. Daptomycin is a member of a group of compounds known as "A-21978C_0 type antibiotics." *See, e.g.*, '238 patent, col. 1:63-66. These compounds share the same "cyclic region" and "tail region" shown in Figure 2. They differ in the length of their fatty acid chains.

20. Daptomycin can be made by fermentation of *S. roseosporus* bacteria. Methods to produce daptomycin by fermenting *S. roseosporus* are discussed throughout the high purity patents. '238 patent, col 9:9-21:40; Examples 1-18. The high purity patents also discuss methods of isolating and purifying daptomycin from fermentation media. *Id.* The high purity patents further discuss other patents that describe fermentation production of daptomycin. '238 patent, col. 1:58-3:46.

V. DAPTOMYCIN IS A NATURAL PRODUCT AND THE MISIDENTIFICATION OF ITS ASPARAGINE STEREOCHEMISTRY WOULD NOT BE SIGNIFICANT TO A PERSON OF ORDINARY SKILL IN THE ART

21. Daptomycin was initially determined to have an L-asparagine ("L-Asn") residue

in its tail portion in the 1980's by Dr. Manuel Debono and his colleagues at Lilly Research Laboratories. Debono et al., *J. Antibiot.*, 40(6):761-777 (1987) (Exhibit 4). Dr. Debono's group used a hydrolysis method to evaluate daptomycin's stereochemistry. *Id.* at 764-772. This was an accepted method at the time. This method converts all of the asparagine amino acids into aspartic acid. Because daptomycin has three L-aspartic acid ("L-Asp") residues in addition to the asparagine amino acid, it would be very difficult (if not impossible) for one skilled in the art to distinguish the D-Asn from the L-Asp when using this methodology. Further, those of ordinary skill in the art in the 1980's understood that chiral amino acids (like asparagine) typically have the L-configuration in peptides. Therefore, it is not surprising that applying technology available in the 1980's, Dr. Debono's group concluded that daptomycin's asparagine residue was L-Asn.

22. In the early 2000's, Miao et al. analyzed the genetic coding for daptomycin using sophisticated techniques that were not available in the 1980's, and concluded that daptomycin has a D-asparagine residue instead of a L-asparagine. Miao et al., *Microbiology*, 151:1507-1523 (2005) (Exhibit 5) at 1512, 1520. In particular, Dr. Miao's group identified genetic material for an epimerase (*i.e.*, a type of enzyme) that converts L-asparagine to D-asparagine, indicating that the asparagine in daptomycin was D-Asn. *Id.* To confirm the stereochemistry, Dr. Miao's group used synthetic chemistry techniques (as opposed to fermentation) to make an L-Asn daptomycin analog as well as D-Asn daptomycin to compare their biological activities. *Id.* at 1520. Dr. Miao's group observed that, tested against the same bacteria, the L-Asn daptomycin analog had 10-fold less activity than daptomycin. *Id.* This result confirmed that the *S. roseosporus* fermentation product, whose activity against bacteria was widely known, had the D-Asn at this

position.

23. The clarification of the D-Asn configuration did not change what daptomycin is. The naturally produced structure of daptomycin was always the same, regardless of the inadvertent misassignment of the stereochemistry of asparagine in the original structural determination by Debono et al.[2] The daptomycin compound produced by fermentation of *S. roseosporus* has only ever been shown to possess D-Asn. As originally described, this antibiotic substance was known as the compound "daptomycin;" after the correction in assignment by Dr. Miao, this compound continued to be known as "daptomycin." The molecule had not changed; this was only a clarification of one structural feature.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct and that this declaration was executed in San Diego, California on February 19, 2013.

Dr. William Gerwick

[2] It is important to consider the limited nature of the misassignment made by Dr. Debono's group. Daptomycin is a large molecule containing 72 carbon atoms and 13 chiral carbons. Using the basic methods of analysis that were available at the time (*i.e.*, HPLC alone as compared to genetic analysis), only one out of those 13 chiral carbons was misidentified as being L rather than D by the Lilly scientists.